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BACTERIAL PUSTULE DISEASE IN SOYBEANS: ARTIFICIAL INOCULATION, VARIETAL RESISTANCE, AND INHERITANCE OF RESISTANCE

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Since this manuscript has been written an article by E. E. Hartwig and S. G. Lehman (Agron. Jour. 43:226-229, 1951) has appeared in which they discuss the inheritance of resistance to the bacterial pustule disease in soybeans. They concluded that the resistance of Clemson Nonshatter is conditioned by a simple recessive factor and that certain other varieties carry modifying genes for partial resistance. Their conclusions are in agreement with those in this paper.

BACTERIAL PUSTULE DISEASE IN SOYBEANS: ARTIFICIAL INOCULATION, VARIETAL RESISTANCE, AND INHERITANCE OF RESISTANCE[1]

CARL V. FEASTER[2]

INTRODUCTION

The bacterial pustule disease of soybeans, caused by *Xanthomonas phaseoli* var. *sojensis* Hedges, Starr and Burkholder, has been the most prevalent disease affecting soybeans in Missouri. The heaviest infections of the disease were observed in the years 1945 and 1949. Over a period of years, it has occurred more intensely in southeast Missouri than in the remainder of the state.

The disease was reported first in South Carolina by Smith (28)[3] in 1904. Hedges (20) observed its occurrence in Texas, Virginia, Arkansas, North Carolina, South Carolina and Louisiana during the early 20's. Recently, it has been reported in all the soybean producing areas of the corn and cotton belts (1, 2, 3, 4, 5, 6). The most severe infections have occurred in the southern states.

Since no resistant varieties, which are adapted to Missouri, are available, an investigation was undertaken to determine the possibility of developing adapted, resistant varieties. This included a study of artificial inoculation, varietal resistance, inheritance of resistance and linkage relations.

The experimental procedures utilized and the results of the experiments are presented in four parts: (1) Determination of the effect of time of inoculation on the severity of infection of soybeans by *Xanthomonas phaseoli* var. *sojensis* Hedges; (2) Determination of varietal resistance of soybeans to infection by *Xanthomonas phaseoli* var. *sojensis;* (3) Inheritance of resistance to *Xanthomonas phaseoli* var. *sojensis* Hedges in soybeans; and (4) Linkage relations between the factors for resistance to infection by *Xanthomonas phaseoli* var. *sojensis* Hedges and maturity.

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[3]See Literature Cited, page 25.

LITERATURE REVIEW

Studies of the various diseases of the soybean indicate that varieties differ in their relative resistance to certain bacterial and fungus diseases. Cromwell (14) in 1919 found, in extensive variety tests, all varieties susceptible to fusarium blight (*Fusarium bulbigenum* Cke. and Mass. var. *tracheiphilum*—E. F. S.—Wr.) with the exception of the variety Black Eyebrow. Woodworth and Brown (30) in 1920 studied the varietal resistance and susceptibility of soybeans to bacterial blight (*Bacterium glycineum* Coerper). Their field experiments indicated that varieties differ greatly in their relative susceptibility to bacterial blight. Of 48 varieties studied, about half were completely resistant, and the other half ranged from complete susceptibility to partial resistance. In contrast, Wolf's (29) studies failed to reveal any evidence of varietal differences in resistance or susceptibility to bacterial blight in any of the varieties observed.

Lehman and Woodside (24) in 1929 studied the resistance and susceptibility of 55 soybean varieties to the bacterial pustule disease. Of these 55 varieties, three were classified as having high resistance. Of the three, the Columbia variety was found to have the greatest resistance. None of the three varieties classified as having high resistance is now available commercially.

Hedges (20) and Lehman and Woodside (24) found that natural infections of the bacterial pustule disease were erratic from location to location and year to year. They found that the disease could be produced artificially by spraying soybean plants with a fine mist of a water suspension of the bacterial pustule organism. In most cases the plants were kept under humid conditions immediately following the inoculations. All these workers indicated that infections obtained artificially were not as severe as infections encountered in field epidemics. Furthermore, they were unable to obtain infections consistently from different inoculations.

Various techniques have been utilized in developing artificial epidemics of different bacterial leaf spot diseases, but the water-soaking technique first used by Clayton (12, 13) on tobacco appears to be the most successful. Clayton has shown that artificial epidemics of wildfire of tobacco could be produced by spraying water-soaked leaves with a water suspension of *Bacterium tabaci*. Johnson (21, 22) confirmed Clayton's results and demonstrated that this situation is not peculiar to tobacco or to the organisms that cause tobacco wildfire or blackfire disease. A number of plant species become equally susceptible to attack by the same organisms when their tissues are water-soaked. Infection was obtained on tomato, alfalfa, bean, pea, hemp, rose, apple, locust, flax, marigold and poinsettia.

Simpson and Weindling (27) were able to produce artificial epidemics of bacterial blight of cotton by the water-soaking technique. They also confirmed Johnson's theory regarding the influence of water-soaking upon host resistance. The Stoneville 20 cotton variety is resistant to bacterial blight under field conditions but becomes slightly infected when artificially inoculated by a strong spray of a water suspension of the organism. The disease symptoms produced by artificially inoculating this variety are readily distinguished from those of the non-resistant strains. More recently, Allington (8) has shown that the wildfire disease of soybeans can be produced by inoculating the leaf tissue with a coarse spray of a water suspension of *Bacterium tabaci*. He stresses the necessity of the existence of a liquid junction between the external and intercellular liquids for the rapid penetration and dispersion of the bacteria. The duration of water-soaking has a limited influence on the multiplication of the bacteria within the host tissue.

In the process of soaking a leaf with a strong spray of water, the water presumably enters the intercellular spaces by way of stomata or leaf injuries. Diachun and Valleau (15) reported that the susceptibility of tobacco leaves to water-soaking by a stream of water was determined largely by the degree of opening of the stomata. In a series of experiments, they found that during the day, the stomata are open and leaves could be water-soaked easily and rapidly. At night, stomata usually are closed or nearly closed, and leaves become water-soaked only with difficulty. Later experiments by Diachun (16) showed that when water suspensions of *Bacterium tabaci* were used in place of plain water in the inoculation experiments, the intensity of infections were in agreement with the intensity of water-soaking of the tissue.

Resistance to many plant diseases has been shown to be inherited; however, its genetic behavior does not follow any one genetic law (7, 9, 10, 11, 17, 18, 25). In some cases, resistance and susceptibility are dependent upon a single factor pair. In other instances resistance and susceptibility are dependent upon multiple factors. Resistance may be either recessive or dominant. Knight (23) has recently made a survey of plant breeding literature to determine the prevalence of major gene control in the genetics of disease resistance. He found in 33 crop plants that major genes were responsible for resistance to 84 pests and diseases. Knight suggests, however, that control by a single powerful major gene accompanied by minor genes is more likely to be a common genetic situation in many economic characters. Therefore, where a character would have been of value in the wild state, a common form of genetic control is likely to be by one or two major genes accompanied by a number of minor genes and modifiers. Resistance to disease is an example of a character that would be of value in the wild state.

EXPERIMENTAL PROCEDURES AND RESULTS

1. Determination of the Effect of Time of Inoculation on the Severity of Infection of Soybeans by *Xanthomonas phaseoli* var. *sojensis* Hedges.[1]

Materials and Methods.—This experiment was conducted during the summer of 1945 using Chief and Lincoln varieties of soybeans growing on the University of Missouri South Experimental Farm, Columbia, Missouri. Four replications of ten rows each of these varieties were planted on June 28. The rows were 10 feet in length and alleys 5 feet in width were left between replications to provide space for operating the spraying equipment.

The organism used in preparing the inoculum was isolated from a typical bacterial pustule lesion on a naturally infected plant of the Chief variety. It was grown on potato dextrose agar slopes and then transferred to sterile water blanks. About 2 or 3 cubic centimeters of this water suspension was poured on agar slopes which had been prepared in 16-ounce medicine bottles. Abundant growth of the organisms was obtained in 48 hours at room temperature. Prior to spraying the plants, the bacteria were removed from the bottles by shaking with water, and the contents of 2 bottles added to 50 gallons of water.

The soybeans were inoculated July 27 at which time the plants averaged 8 to 10 inches in height. One row from each replication was inoculated each 2-hour period throughout the day starting at 3 a. m. Central Standard Time and continuing until total darkness at 9 p. m. The inoculum was applied with a power sprayer, maintaining approximately 150 pounds pressure. The nozzle was held about 30 inches from the rows and was adjusted to deliver an unbroken stream of water until within a few inches of the leaves, where the spray broke into large droplets similar to a beating rain. The rows were sprayed from both sides so that more inoculum could be brought in contact with the under leaf surface where most of the stomata are found.

Strips of the lower leaf epidermis were removed and placed into 100 per cent alcohol at the time of each inoculation. From these, the average width of the stomatal openings was later determined. Another check of the stomatal openings were made by dropping alcohol, toluene and xylene on the lower leaf surfaces and noting the amount of penetration by each liquid.

Disease notes were taken 10 days after inoculation. The severity of infection for each row was recorded on a scale of 0 to 10, no infection being indicated by zero and heavy infection on all leaves being indicated by a rating of 10.

[1]This phase of the work has been published previously in part, jointly with W. B. Allington. The Relation of Stomatal Behavior at the Time of Inoculation to the Severity of Infection of Soybeans by *Xanthomonas phaseoli* var. *sojensis* Hedges (Starr) Burk. Phytopathology 36:385-386, 1946.

Results.—The results show that the time of day greatly influences the size of the stomatal openings and the amount of infection obtained. Figure 1 illustrates differences in the amount of infection and shows the correlation with stomatal behavior. During the bright part of the day, 7 a. m. to 1 p. m., the

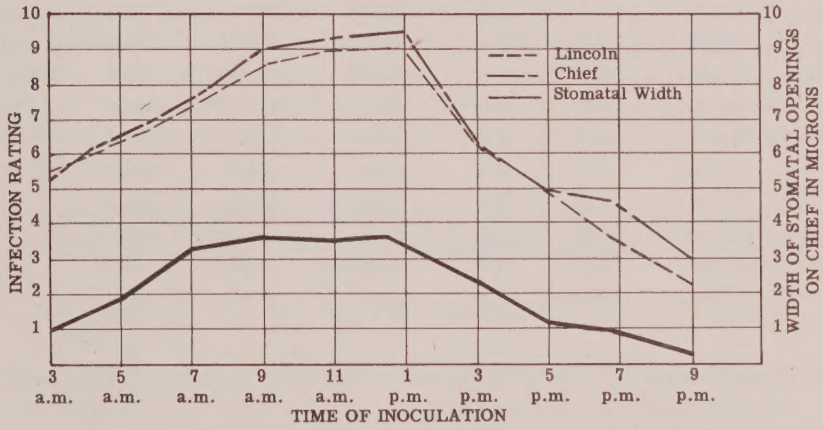

Figure 1. Correlation of stomatal behavior and infection ratings on Chief and Lincoln soybeans when inoculated by power spraying at different times of the day.

stomatal openings were the widest and the most severe infection resulted; while during the early morning and late evening the stomata were closed or only partially open and less infection occurred. At 3 a. m. and 9 p. m. little infection resulted.

Water-soaking of the leaf tissue by the spray operation was apparent immediately after inoculations during the bright part of the day, but was inconspicuous after inoculations made at other times. This agrees with the observations of Diachun and Valleau (15).

The use of alcohol, toluene and xylene provides a rapid and effective means of determining the degree of stomatal opening. Table 1 shows the measurement of the stomata at various times of the day as compared to the penetration of each of the liquids and the degree of infection. The penetration of toluene and xylene was identical; however, alcohol penetrated less readily than these liquids in early morning and late afternoon. Heavy infection was associated with rapid penetration, and light infection was associated with little or no penetration.

These results show that the most severe infections were obtained with the inoculations made at the time of day when stomatal openings were the greatest. It would appear therefore that the bacteria enter more freely through the open stomata. Heavy infections resulted when the stomata were open sufficiently to make possible the rapid penetration of alcohol.

Table 1. Measurement of the Stomata at Various Times of the Day as Compared to the Penetration of Each of the Liquids, and the Degree of Infection Obtained.

Time of Inoculation	Infection Rating	Stomata Width (microns)	Penetration		
			Alcohol	Toluene	Xylene
3 a.m.	5.3	1.0	none	none	none
5 a.m.	6.5	1.9	none	slight	slight
7 a.m.	7.5	3.4	slight	good	good
9 a.m.	9.0	3.6	good	good	good
11 a.m.	9.3	3.5	good	good	good
1 p.m.	9.5	3.7	good	good	good
3 p.m.	6.3	2.3	slight	good	good
5 p.m.	5.5	1.2	slight	fair	fair
7 p.m.	4.5	0.9	none	slight	slight
9 p.m.	3.0	0.4	none	none	none

2. **Determination of Varietal Resistance of Soybeans to Infection by *Xanthomonas phaseoli* var. *sojensis* Hedges.**

Field Studies. *Materials and Methods.*—Forty-eight varieties, varying widely in maturity, were compared for resistance to the bacterial pustule disease. Seed of these varieties was obtained from the United States Department of Agriculture and various state agricultural experiment stations. They were planted at Columbia, Missouri, June 14, 1945 in a randomized complete block. Each variety was planted in a row 10 feet in length and appeared once in each of the four replications. Alleys 5 feet in width were left between each of the replications to provide space for operating the spraying equipment. The plants were 8 to 10 inches in height when they were inoculated.

The inoculum was prepared in the same manner as in part one of this study. Approximately 50 gallons of inoculum was applied to each replication. This amount was prepared prior to the inoculation of each replication so that each variety within the replication would receive the same concentration of bacteria. The inoculum was applied between 7 a. m. and 1 p. m. on July 17 with a power sprayer in the same manner as in the experiment of part one. The degree of opening of the stomata was determined prior to the inoculation of each replication by noting the degree of penetration of alcohol, xylene and toluene. In all cases the three liquids penetrated readily, indicating the stomata were open sufficiently to permit entrance of the inoculum.

Results.—Symptoms appeared on most varieties within six days after inoculation. With the exception of one variety, Clemson Nonshatter, all or practically all the plants of each variety in the test were infected. The lesions produced were characterized by the development of a brownish pustule surrounded

by a yellow halo. As the disease advanced the pustules ruptured and a reddish-brown spot appeared surrounded by a yellow halo.

Very few lesions were present on the plants of the Ogden variety, indicating moderate resistance. The varieties Boone and Harman were most heavily infected, and the amount of infection on the other varieties approached that on Boone and Harman. Figure 2 shows the appearance of typical leaves from the varieties Clemson Nonshatter and Boone 10 days after inoculation. To obtain a basis for comparing resistance, the varieties were divided into three groups: (a) resistant, including only Clemson Nonshatter; (b) moderately resistant, including only Ogden, and (c) susceptible, including the remainder of the varieties.

Table 2. Infection Ratings for the Varieties Included in the Varietal Resistance Test at Columbia, 1945. Infection Ratings Were Made on a Scale of Zero to Ten.

	Infection Rating		Infection Rating
Easycook	4.3	Volstate	4.8
Osoya	5.0	Delsoy	4.3
Higan	4.0	Clemson Nonshatter	0.0
Imperial	4.5	Laredo	3.8
Willomi	5.0	Ito San	4.0
Hahto	5.8	Wilson	4.5
Fuji	4.0	Kingwa	4.8
Aoda	5.8	Ottowa Mandarin	5.0
Rokusun	5.0	Habaro	5.0
Mamredo	6.3	Herman	4.5
Dunfield	6.5	Kabott	5.0
Armredo	4.5	Ontario	5.5
Ogden	1.3	Goldsoy	5.0
Arksoy 2913	5.3	Cayuga	4.0
Illini	6.0	Minsoy	5.5
Viking	5.0	Gibson	6.5
Harman	6.8	Macoupin	6.5
Richland	4.8	Boone	6.8
Mukden	6.3	Chief	4.5
Wisc. Manchu 3	5.3	Lincoln	5.8
Mingo	5.5	S-100	6.3
Earlyana	6.0	Ralsoy	5.8
Scioto	5.5	Patoka	4.8
Rose Non-Pop	4.8	Magnolia	5.3

On the twelfth day, lesions began to develop on the Clemson Nonshatter variety. The lesions were sparse and were not typical of those found on the other varieties. They were characterized by the development of dark angular spots without pustular outgrowths, and in most cases without a halo. Figure 3 shows the typical lesions as they appeared on Lincoln, a susceptible variety, and Figure 4 shows the lesions typical of those found on Clemson Nonshatter.

Figure 2. Typical leaves from the varieties Clemson Nonshatter (top) and Boone (bottom) ten days after inoculation. Lesions are absent on the Clemson Nonshatter variety but numerous on the Boone variety.

Isolation of *Xanthomonas phaseoli* var. *sojensis* Hedges from the lesions confirmed the identity of this disease.

In contrast to the above observation, no lesions developed on the Clemson Nonshatter variety under natural conditions. Apparently, the resistance of Clemson Nonshatter is lowered sufficiently by water-soaking to permit the development of lesions when artificially inoculated.

Greenhouse Studies. *Materials and Methods.*—During the winter of 1945-46 seed from each of the following varieties was planted in 3-gallon pots in the greenhouse at Columbia, Missouri: Clemson Nonshatter, Macoupin, Richland, Aoda, Ogden, Lincoln, Magnolia, Chief and Boone. These varieties represent a range in reaction to the bacterial pustule disease from the resistant to

the most susceptible, as indicated by the field tests. After the plants had attained suitable size, they were artificially inoculated. A padded board was held against the upper surface of each leaf to be inoculated, and a stream of inoculum from a power sprayer was directed toward the exposed under surface. At first, the stream was adjusted to allow a more or less solid column for about 5 inches, at this point the column gradually broke into a spray of drops. The nozzle was held 8 to 10 inches from the leaf surface until a water-soaked condition developed. Within three days, injury resulting from the beating of the sprayed water against the tissue had become so severe that it was impossible to take infection readings. The water-soaked condition was no more prevalent during this inoculation than had been encountered in the field experiments, but injury was more severe due to the leaf tissue being more tender.

Figure 3. Typical lesions as they appeared on Lincoln fourteen days after inoculation. The lesions are characterized by the development of pustular outgrowths, the base of which is circumscribed by a yellow halo.

Figure 4. Typical lesions as they appeared on Clemson Nonshatter fourteen days after inoculation. The lesions are dark brown angular spots with no pustular outgrowths. The characteristic yellow halo normally found at the base of the pustule was either limited or not present.

Two weeks later, 25 leaves of each variety were again inoculated. Less pressure was used and a rotary disc was placed in the nozzle to prevent the formation of a solid stream. The resulting stream consisted mostly of small droplets. The spray was directed against the under surface of the leaf until small patterns of water soaked tissue appeared throughout the leaf. Lesions appeared within four days; however, rating of intensity of the infection on the varieties was not attempted until two days later. Macoupin, Aoda, Magnolia and Chief were severely infected; Richland, Dunfield, Lincoln and Boone showed moderate infection; and slight infection was present on Clemson Nonshatter and Ogden. Twelve days after inoculation, little or no difference was noted in the number of lesions present on the different varieties, but the same distinction in the appearance of the lesions on Clemson Nonshatter as was exhibited under field conditions, was again present.

All varieties were more susceptible under greenhouse conditions than in the field. This difference was relatively greater in the most resistant hosts. The rates at which they became infected was the most obvious difference among varieties, except in the case of the Clemson Nonshatter variety.

It is apparent that under greenhouse conditions the inoculum must be applied with less pressure and for a shorter duration than in the field. Otherwise, severe water-soaking injury will result and interfere with the reading and interpretation of the resulting infections.

3. Inheritance of Resistance to *Xanthomonas phaseoli* var. *sojensis* Hedges in Soybeans.

Materials and Methods.—Clemson Nonshatter, Ogden, Boone and Lincoln varieties were employed in this study. Clemson Nonshatter is resistant to the bacterial pustule disease, Ogden is moderately resistant, and Boone and Lincoln are susceptible. Clemson Nonshatter is commonly grown in the southern cotton belt and Ogden in the northern cotton belt. Ogden constitutes approximately 40% of the acreage in southeast Missouri, but Clemson Nonshatter is entirely too late maturing to be grown in Missouri. Lincoln is adapted to northern Missouri and Boone is adapted to central Missouri. Lincoln, Boone and Ogden each were crossed with Clemon Nonshatter and the six possible backcrosses were made. The F_1 generation was used in the backcrosses. This permitted the crossing of Clemson Nonshatter with varieties of three different maturity groups. Lincoln requires approximately 110 days to mature. Boone 125 days, and Ogden 145 days. Clemson Nonshatter requires approximately 160 days to mature.

Figure 5 shows the typical lesions as they appeared on each of the four varieties.

The amount of natural crossing in soybeans has been found negligible by several workers (19, 26, 31); therefore, it is reasonable to assume a relatively homozygous condition for the gene complexes of the varieties employed in this study.

The method of preparing the inoculum and the inoculation technique were similar to that previously described. The severity of infection was estimated on a scale of 0 to 3 as follows:

0: Lesions with no pustular outgrowths.

1: Lesions sparse with small pustular outgrowths.

2: Moderate number of lesions with pustular outgrowths.

3: Numerous lesions with pustular outgrowths.

Readings of 0 designate resistance; 1 through 3, susceptibility.

Under artificial inoculation, Boone has an average index of 3, Lincoln 2, Ogden 1.5 and Clemson Nonshatter 0. With natural infection, the relative intensities of infection are similar.

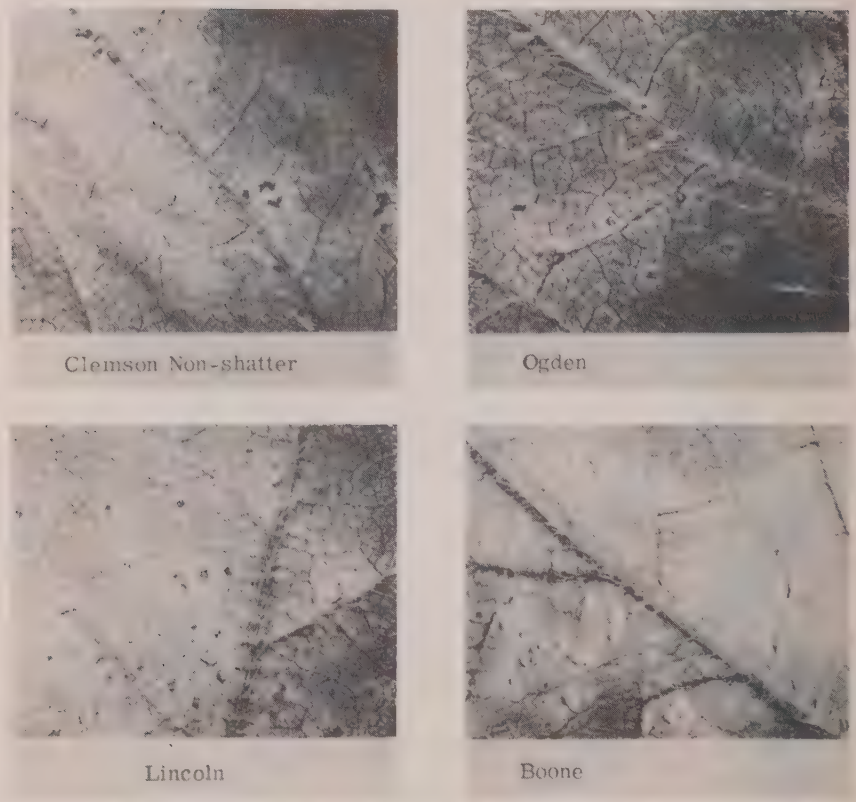

Figure 5. Typical lesions as they appeared on each of the four parent varieties ten days after inoculation.

Results.—Clemson Nonshatter x Lincoln and Backcrosses: Twelve F_1 plants were grown and inoculated in the field in 1947 and thirteen in 1948. All the F_1 plants were as susceptible as the Lincoln variety, thus indicating complete dominance for susceptibility. The F_1 plants were given a rating of 2.

The F_2 generations of this cross were grown in the field both during 1948 and 1949. Table 3 gives the results of the inoculation of 719 plants in the field in 1948 and 266 plants in 1949. The results for each F_2 family are included. Deviation occurs, in some families, from any constant ratio; however, several families show a close approximation to a 3:1 ratio between susceptibility and resistance with susceptibility dominant. These deviations can be expected with small numbers such as occur in individual families. The totals of all the families give a close approximation of a 3:1 ratio. Of the families grown in 1948, the total number of resistant plants deviated only 11.75 plants from the expected.

Table 3. Results from Inoculating F_2 Plants of Clemson Nonshatter x Lincoln in Field, 1948-49.

Pedigree No. of F_1 Plants	No. of F_2 Plants 1948		Pedigree No of F_1 Plants	No. of F_2 Plants 1949	
	Res.	Susc.		Res.	Susc.
A-1-1	32	67	A-1-13	2	15
A-1-2	26	75	A-1-14	7	14
A-1-3	8	28	A-1-15	4	13
A-1-4	15	49	A-1-16	6	18
A-1-5	15	63	A-1-17	1	21
A-1-6	9	39	A-1-18	3	21
A-1-7	12	38	A-1-19	5	15
A-1-8	14	57	A-1-20	6	14
A-1-9	6	34	A-1-21	4	17
A-1-10	9	27	A-1-22	2	21
A-1-11	10	38	A-1-23	4	15
A-1-12	12	36	A-1-24	6	14
			A-1-25	6	12
Totals	168	551		56	210
Expected Numbers	179.75	539.25		66.50	199.50
Difference	11.75 ± 11.612			10.50 ± 6.982	
Probability	0.32			0.14	

Table 4. Results from Inoculating F_2 Plants of Clemson Nonshatter x Boone in Greenhouse, 1948-49, and Field, 1949.

Pedigree Number of F_1 Plants	No. of F_2 Plants in Greenhouse		No. of F_2 Plants in Field		Total No. of F_2 Plants	
	R	S	R	S	R	S
B-1-1	8	16	9	26	17	42
B-1-2	6	17	6	22	12	39
B-1-3	4	19	6	34	10	53
B-1-4	7	17	6	34	13	51
B-1-5	6	18	13	30	19	48
B-1-6	7	17	15	24	22	41
B-1-7	8	15	8	31	16	46
B-1-8	7	14	7	17	14	31
B-1-9	6	16	10	31	16	47
B-1-10	6	17	11	29	17	46
B-1-11	4	20	16	25	20	45
B-1-12	6	18	14	32	20	50
B-1-13	5	17	10	31	15	48
B-1-14	8	16	8	29	16	45
B-1-15	2	21	7	37	9	58
B-1-16	3	15	6	31	9	46
B-1-17	5	15	5	44	10	59
Totals	98	288	157	507	255	795
Expected Numbers	96.50	289.50	166.00	498.00	262.50	787.50
Difference	1.50 ± 8.509		9.00 ± 11.158		7.50 ± 14.037	
Probability	0.86		0.42		0.60	

The results of the F_2 generation grown in 1949 (Table 3) also gave a ratio of approximately three susceptible to one resistant progeny. Of 266 F_2 plants which were inoculated, 56 showed resistance and 210 susceptibility to bacterial pustule. This ratio indicates that resistance to bacterial pustule in this cross behaved in segregation as a single unit factor with susceptibility being dominant.

One hundred of the F_2 plants grown in 1948 were selected at random and grown in plant rows in 1949. Approximately 25% of these third generation families bred true for resistance, 50% gave both resistant and susceptible plants, and approximately 25% bred true for susceptibility. The actual result by families were as follows: 25 homozygous resistant, 48 heterozygous, and 27 homozygous susceptible. All resistant families were from F_2 plants which were classified as resistant. The heterozygous and susceptible families were from susceptible F_2 plants.

To bear out the 3:1 hypothesis the heterozygous F_3 families should give the same general segregation as the families of the F_2 generation. Here also many of the individual families as well as the totals for the families approximated very closely a 3:1 ratio with susceptibility being dominant. The total number of resistant plants deviated only 13 plants from the expected number (Table 6).

Table 5. Results from Inoculating F_2 Plants of Clemson Nonshatter x Ogden in Greenhouse, 1948-49, and Field, 1949.

Pedigree Number of F_1 Plants	No. of F_2 Plants in Greenhouse		No. of F_2 Plants in Field		Total No. of F_2 Plants	
	R	S	R	S	R	S
C-1-1	8	19	14	25	22	44
C-1-2	1	26	14	25	15	51
C-1-3	9	19	9	38	18	57
C-1-4	8	18	13	21	21	39
C-1-5	6	20	7	21	13	41
C-1-6	5	21	8	24	13	45
C-1-7	10	18	11	38	21	56
C-1-8	7	15	7	18	14	33
C-1-9	6	22	10	27	16	49
C-1-10	4	22	10	34	14	56
C-1-11	8	19	6	32	14	41
C-1-12	6	21	11	21	17	42
C-1-13	8	17	15	29	23	46
C-1-14	4	22	5	31	9	53
C-1-15	1	7	8	23	9	30
Totals	91	286	148	407	239	693
Expected Numbers	94.25	282.75	138.75	416.25	233	699
Difference	3.25 ± 8.407		9.25 ± 10.201		6.00 ± 13.219	
Probability	0.70		0.36		0.65	

The progeny from the backcross Lincoln x (Clemson Nonshatter x Lincoln) were all susceptible (Table 7). This is evidence that susceptibility is inherited as a dominant factor. The progeny from the backcross Clemson Nonshatter x (Clemson Nonshatter x Lincoln) were 50% resistant and 50% susceptible (Table 8). From a total of 36 plants, 18 were rated as resistant and 18 as susceptible. This substantiates the previous results.

Since Lincoln is slightly less susceptible than Boone, it is possible that Lincoln may have a few secondary genes for resistance. Some variation occurred among the susceptible F_2 segregates; however, it was difficult to determine whether the variation was the result of genetic differences or differences in the inoculation of individual plants. Of the 761 plants classified as susceptible, 12 were rated as 1, 354 as 2 and 395 as 3. The variation was greater among the susceptible F_2 plants of this cross than of Clemson Nonshatter x Boone cross but not as pronounced as in the Clemson Nonshatter x Ogden cross.

The above results indicate that the resistance of Clemson Nonshatter is conditioned by a simple recessive factor. Lincoln may possibly possess a few secondary genes for resistance which make it slightly less susceptible than Boone.

Clemson Nonshatter x Boone and Backcrosses: Seventeen F_1 plants of Clemson Nonshatter x Boone were grown and inoculated in the field in 1948. All plants were susceptible with a rating of 3. The intensity of infection was similar to that obtained on Boone, thus indicating complete dominance for susceptibility.

The F_2 generations were grown and inoculated in the greenhouse in 1948-49 and in the field in 1949. Of the 386 F_2 plants grown in the greenhouse, 98 were classified as resistant (Table 4). This deviates only 1.5 plants from a 3:1 ratio of susceptible to resistant plants. Table 4 also shows the results from the inoculation of 664 F_2 plants in the field in 1949. These F_2 plants were from

Table 6. Results from Inoculating the Following F_3 Families Heterozygous for Disease Resistance: (1) Clemson Nonshatter x Lincoln, (2) Clemson Nonshatter x Boone, and (3) Clemson Nonshatter x Ogden.

	Clemson Nonshatter x Lincoln		Clemson Nonshatter x Boone		Clemson Nonshatter x Ogden	
	Res.	Susc.	Res.	Susc.	Res.	Susc.
Observed Numbers	394	1234	216	697	167	568
Expected Numbers	407.00	1221.00	228.25	684.75	183.75	551.25
Difference	13.00 ± 17.471		12.25 ± 13.083		16.75 ± 11.739	
Probability	0.46		0.35		0.15	

the same F_1 plants as the F_2 plants grown in the greenhouse. The results of each individual F_2 family are included. These results were similar to those obtained in the greenhouse. Approximately three-fourths of the plants were susceptible and one-fourth were resistant. The actual number of resistant plants differed by 9 from the expected number. The combined results of inoculating the F_2 plants of the Clemson Nonshatter x Boone cross in the greenhouse and field gives a total number of resistant plants that is 7.5 less than the expected if resistance is inherited as a simple, recessive factor.

Progenies from 100 plants, selected from those grown in the greenhouse, were grown in plant rows in 1949. Since many of the F_2 plants grown in the greenhouse bore very few seeds, the 100 plants selected were those bearing the most seed. Of these F_3 families, 32 bred true for resistance, 46 families were heterozygous for resistance, and 22 families bred true for susceptibility. This deviates somewhat from the theoretical of 25 homozygous resistant, 50 heterozygous and 25 homozygous susceptible; however, a deviation this great can be expected by chance 20 to 30 times out of 100. With no exceptions, the resistant F_3 families were from resistant F_2 plants. The heterozygous and susceptible families were from susceptible F_2 plants.

There was a total of 913 plants in the 46 heterozygous F_3 families. Of these, 216 plants were resistant (Table 6). This number deviates 12.25 plants from the expected 3:1 ratio of susceptible to resistant plants. The progeny from the backcross Boone x (Clemson Nonshatter x Boone) were all susceptible (Table 7). The progeny from the backcross Clemson Nonshatter x (Clemson Nonshatter x Boone) gave 8 resistant and 9 susceptible plants (Table 8); thus, further supporting the hypothesis that resistance is inherited as a simple, recessive factor.

Table 7. Results from Inoculating BC_1 Plants from the Backcrosses with the Susceptible Parent.

	Resistant		Susceptible	
	Actual	Expected	Actual	Expected
Lincoln x (Clemson Nonshatter x Lincoln)	0	0	140	140
Boone x (Clemson Nonshatter x Boone)	0	0	156	156
Ogden x (Clemson Nonshatter x Ogden)	0	0	213	213

No variation in infection among the susceptible F_2 plants was noted in excess of that expected by differences in the inoculation of individual plants. Of the 795 plants classified as susceptible, seven were classified as 1, 126 as 2 and 662 as 3. It is likely that Boone carries no secondary factors for resistance.

Table 8. Results from Inoculating BC_1 Plants from the Backcrosses with the Resistant Parent.

	Resistant		Susceptible		
	Actual	Expected	Actual	Expected	Probability
Clemson Nonshatter x (Clemson Nonshatter x Lincoln)	18	18	18	18	
Clemson Nonshatter x (Clemson Nonshatter x Boone)	8	8.5	9	8.5	0.81
Clemson Nonshatter x (Clemson Nonshatter x Ogden)	33	35	37	35	0.63

Clemson Nonshatter x Ogden and Backcrosses: It has been pointed out that Ogden is partially resistant. The pustules are smaller and less numerous than on the Lincoln or Boone varieties. Fifteen F_1 plants from the cross Clemson Nonshatter x Ogden were grown and inoculated in the field in 1948. The lesions appeared similar to those found on Ogden. In comparison with the F_1 plants of Clemson Nonshatter x Lincoln and Clemson Nonshatter x Boone, the lesions on the F_1 plants of Clemson Nonshatter x Ogden were fewer, smaller and slower to develop. Definite pustules did, however, develop, thus indicating that the resistance of Clemson Nonshatter is recessive.

The F_2 generations were grown and inoculated in the greenhouse in 1948-49 and in the field in 1949. Of the 377 F_2 plants grown in the greenhouse, ninety-one were classified as resistant (Table 5). This deviates 3.25 plants from a 3:1 ratio. Plants were classified as resistant if no pustular outgrowths were present. There was a gradation in susceptibility among the F_2 plants as indicated by size and number of pustules. Of the 286 plants classified as susceptible 66 were rated as 1, 142 as 2 and 78 as 3. This indicates that secondary factors for resistance are also present in this cross. There was some indication of a variation among the plants classified as resistant; however, this would be difficult to verify. The flecks, resistant type lesions, were, on some plants, very small and sparse in spite of evidence of heavy water-soaking of the leaf tissue.

Table 5 also shows the results of the inoculation of 555 F_2 plants in the field in 1949. The plants were from the same F_1 plants as the F_2 plants grown in the greenhouse. The results were similar to those obtained from the greenhouse test. The actual number of resistant plants deviated 9.25 from the expected 3:1 ratio of susceptible to resistant. Here again, a variation among the susceptible plants was evident, not only in size and number of pustules but also in rate of development. Many F_2 plants were much more susceptible than the

less resistant parent, Ogden. Of the 407 susceptible plants, 99 were classified as 1, 210 as 2 and 98 as 3. No definite ratio existed among susceptible plants, and thus a multiple factor hypothesis probably best explains the variation among the susceptible plants. The combined results of the inoculation of the F_2 plants of the Clemson Nonshatter x Ogden cross in the greenhouse and the field give a total number of resistant plants that is six less than the expected if resistance is inherited as a simple, recessive factor.

Progenies from 100 plants, selected from those grown in the greenhouse, were grown in plant rows in 1949. In some cases the number of plants in each family was rather small. Of the 100 families, 31 were homozygous resistant, 45 heterozygous, and 24 homozygous susceptible. The resistant F_3 families were from resistant F_2 plants. The heterozygous and susceptible families were from susceptible F_2 plants.

From the results obtained in the F_2 generation, a 3:1 ratio of susceptible to resistant plants should have appeared among the heterozygous F_3 families. Of the 735 plants in the 45 heterozygous families, 167 were classified as resistant (Table 6). This number of resistant plants deviated 16.75 from the expected on the basis of the 3:1 hypothesis.

The progeny from the backcross Ogden x (Clemson Nonshatter x Ogden) were all susceptible (Table 7). The degree of susceptibility was variable, with the majority reacting similarly to Ogden. Some plants were very susceptible and others were less susceptible than Ogden. Of these plants, 57 were rated as 1, 127 as 2 and 29 as 3. This further indicates secondary factors for resistance in addition to the factor for resistance carried by Clemson Nonshatter. The progeny from the backcross Clemson Nonshatter x (Clemson Nonshatter x Ogden) were approximately 50% resistant and 50% susceptible (Table 8). From a total of 70 plants, 33 were classified as resistant and 37 as susceptible. Similar variation existed among the susceptible plants as was noted in the backcross Ogden x (Clemson Nonshatter x Ogden). Of the susceptible plants, 12 were rated as 1, 22 as 2 and 3 as 3.

These results indicate that the major gene for resistance carried by Clemson Nonshatter does not give complete expression of resistance. This is in agreement with the theory expressed by Knight (23). He believes that genes which by themselves give complete expression to a character are rare. Segregates carrying the major factor for resistance from Clemson Nonshatter and minor factors from Ogden should be more resistant than either parent.

4. Linkage Relations Between the Factors for Resistance to Infection by *Xanthomonas phaseoli* var. *sojensis* Hedges, and Maturity.

The F_2 progenies from the cross Lincoln x Clemson Nonshatter and Boone x Clemson Nonshatter were used for studying linkage between the factors for

resistance and maturity. The F_2 population of the Ogden x Clemson Nonshatter cross was too late maturing to allow a similar study. The data for the comparison of the frequencies of resistant and susceptible F_2 progenies within the various maturity classes are presented in Tables 9 and 10.

Table 9. F_2 Segregation for Maturity of Resistant and Susceptible Plants of Clemson Nonshatter x Lincoln, 1948.

Maturity Class	Actual		Expected	
	Susceptible	Resistant	Susceptible	Resistant
9/8-9/14	1	1	1.50	0.50
9/15-9/21	11	3	10.50	3.50
9/22-9/28	17	5	16.50	5.50
9/29-10/5	36	13	36.75	12.25
10/6-10/12	67	24	68.25	22.75
10/13-10/19	116	40	117.00	39.00
10/20-10/26	167	43	157.50	52.50
10/27-11/2	90	27	87.75	29.25
11/3-11/9	24	7	23.25	7.75
11/10------	19	4	17.25	5.75
Unclassified	3	1	3.00	1.00
Probability 0.99				

Table 10. F_2 Segregation for Maturity of Resistant and Susceptible Plants of Clemson Nonshatter x Boone, 1949.

Maturity Class	Actual		Expected	
	Susceptible	Resistant	Susceptible	Resistant
9/22-9/28	5	2	5.25	1.75
9/29-10/5	14	6	15.00	5.00
10/6-10/12	23	7	22.50	7.50
10/13-10/19	20	11	23.25	7.75
10/20-10/26	86	30	87.00	29.00
10/27-11/2	170	49	164.25	54.75
11/3-11/9	36	12	36.00	12.00
11/10-11/16	29	8	27.75	9.25
11/16	124	31	116.25	38.75
Probability .80 - .90				

The frequency distribution of the F_2 plants in each maturity class indicates that maturity was controlled by multiple factors in the cross Lincoln x Clemson Nonshatter. A few plants matured approximately as early as Lincoln and a few matured about as late as Clemson Nonshatter; however, the majority were intermediate in maturity. Within each maturity class, a 3:1 ratio of susceptible and resistant plants was closely approached. This indicated that no linkage relations existed between the factors for maturity and resistance.

Segregation for maturity in the F_2 population of the Boone x Clemson Nonshatter cross was similar to that observed in the Lincoln x Clemson Nonshatter cross, indicating that maturity in this cross was also controlled by multiple factors. The large number of plants included within the latest class was

a result of a killing frost which prevented more detailed classification of these plants (Table 10). It will be noted that the F_2 population of the Lincoln x Clemson Nonshatter cross was grown in 1948 and the F_2 population of the the Boone x Clemson Nonshatter cross in 1949. This accounts for the difference in maturity of the latest plants of the two crosses. No F_2 plants of either cross appeared to be later than Clemson Nonshatter when grown under similar conditions.

The 3:1 ratio of susceptible to resistant plants in each maturity class that was observed in the Lincoln x Clemson Nonshatter cross was observed also in the Boone x Clemson Nonshatter cross. This indicates that earliness and resistance are inherited independently. Since no linkage is evident, it appears that no difficulty should be encountered in obtaining resistant plants of the desired maturity.

DISCUSSION

With the exception of Ogden, all of the varieties commonly grown in Missouri are susceptible to the bacterial pustule disease. Since natural infections are inconsistent year after year and quite variable in reference to location, their use is undesirable as a means of determining the relative resistance of varieties or strains to the disease.

Difficulty in obtaining consistent infections artificially with bacterial leaf spots of field grown crops has been a common experience. Therefore, the testing of varieties for disease resistance frequently requires reliable methods of artificial inoculation in order to be certain of the interpretation of the results. The success in the development of artificial epidemics of wildfire in tobacco (12) has pointed the way for continuing studies with other crops. In the experiments reported herein, artificial epidemics of the bacterial pustule disease of soybeans were consistently produced by inoculating the plants with a strong spray of a water suspension of the organism. The ability to produce infections in epidemic proportions makes possible the reliable testing of soybean varieties for resistance to this disease. Also, it makes possible the testing of hybrid populations in inheritance studies and developing resistant varieties.

There was a close correlation between the size of the stomatal openings and the amount of infection obtained. Differences in stomatal openings at the time of inoculation may result in erroneous conclusions regarding the resistance or susceptibility of plants unless care is taken to make all inoculations when the stomata are open. It is possible to determine whether or not the stomata are open by noting the degree of penetration of alcohol, xylene or toluene. This method is adaptable to use in the field; whereas, actual measurement of the stomata is time consuming and is not adapted to field use.

In a test in which forty-eight varieties were inoculated, one, Clemson Nonshatter, was found to be resistant and one, Ogden, to be moderately resistant.

Lesions developed more slowly and were fewer in number than on the susceptible varieties. Lesions on the Clemson Nonshatter variety were, in addition, distinctly different in appearance from those on Ogden and the susceptible varieties.

The results of the inheritance study indicate that resistance to the bacterial pustule disease of the soybean is inherited in a Mendelian manner. Clemson Nonshatter, a resistant variety, was crossed with the varieties Boone, Lincoln and Ogden. The Boone variety is extremely susceptible, Lincoln is slightly less susceptible and Ogden is moderately resistant. The results obtained from the F_2 generations of these crosses and the BC_1 generations of the six possible backcrosses reveal that the resistance of Clemson Nonshatter is conditioned by a simple, recessive factor. In the Clemson Nonshatter x Lincoln and Clemson Nonshatter x Ogden crosses, multiple secondary factors for resistance were also evident. Apparently, these secondary factors for resistance were contributed by Lincoln and Ogden and account for their being less susceptible than Boone. A few F_2 segregates from these two crosses appeared to be more resistant than either parent. This was especially true in the Clemson Nonshatter x Ogden cross.

Under field conditions Clemson Nonshatter does not develop lesions; therefore, it would be impossible to distinguish strains carrying greater resistance. With artificial inoculation, resistance is lowered sufficiently by water-soaking to allow development of non-typical lesions on Clemson Nonshatter, and on even more resistant segregates. In comparison with the lesions on Clemson Nonshatter, the lesions on the plants classified as highly resistant were extremely small and sparse.

The factors for maturity and resistance appeared to be inherited independently. There should be no difficulty encountered in obtaining resistant plants of the desired maturity if the proper parent material is selected.

When the mode of inheritance is known, the proper breeding program can be more easily initiated. The object of breeding by hybridization is to combine into a single variety the desirable characters of two or more varieties. Occasionally, the recombination of genetic factors results in the production of new and desirable characters not found in either parent. Selection of parents that are relatively satisfactory for these characters will, however, increase the probability of obtaining the desired combination of characters.

It appears logical in breeding for resistance to the bacterial pustule disease that a large F_2 population should be grown. This should permit the elimination of susceptible plants and leave a sufficient number of resistant plants for selection of the desired agronomic characters. Resistance is inherited as a simple, recessive factor, thus all resistant F_2 plants would breed true for resistance. Since Clemson Nonshatter has no desirable characters except resistance to bacterial pustule, it can be expected to contribute little to a cross

in addition to resistance. Other desirable characters must be obtained from the other parent. Practically all of the F_3 lines obtained to date have possessed one or more of the undesirable characteristics of Clemson Nonshatter. Most of the lines are too late maturing for Missouri, and, in general, the early, resistant strains are definitely inferior to the present varieties of similar maturity. This being the case the single cross seems inadequate for developing adapted, resistant varieties.

Another approach to this problem of breeding adapted varieties resistant to the bacterial pustule disease is the backcross. It is a logical procedure when it is desired to add a character, which is easily observed and simply inherited, to an otherwise desirable variety. It is based on the fact that a heterozygous population backcrossed to a homozygous parent will become homozygous for the genotype of the recurrent parent. The proportion of homozygous individuals in any backcross generation is the same as would result from an equal number of selfed generations. The objection to using this method is the improbability of developing a variety superior to the recurrent parent except for the addition of resistance to the bacterial pustule disease.

The disadvantages of the single and backcross methods may be eliminated by using a multiple cross. That is, crossing the best resistant strains with other experimental strains, now available, which possess a combination of desirable characteristics but lack resistance.

SUMMARY

1. A technique for inoculating soybeans with *Xanthomonas phaseoli* var. *sojensis* Hedges is described.
2. Stomatal openings were widest from 7 a. m. to 1 p. m., as shown by two methods of measurement.
3. Water-soaking was greatest and maximum infection with the bacterial pustule disease was obtained when the plants were sprayed at the time the stomata were open the widest.
4. Clemson Nonshatter was the most resistant variety tested. Ogden was moderately resistant, and all other varieties were susceptible.
5. The lesions developed more slowly on Clemson Nonshatter and Ogden and the lesions on Clemson Nonshatter differed in appearance from those on the other varieties.
6. The inheritance of resistance to the bacterial pustule disease was studied in soybean hybrids. The varieties Lincoln, Boone and Ogden were each crossed with the resistant variety, Clemson Nonshatter. The F_1 progenies of these three crosses were backcrossed with their respective parents.

7. Resistance was indicated by the development of lesions without pustular outgrowths, when the segregates were inoculated artificially with a strong spray of a water suspension of the bacterial pustule organism. This type of lesion is identical with that found on Clemson Nonshatter under similar conditions. The susceptible segregates develop lesions with pustular outgrowths.
8. The resistance of Clemson Nonshatter is conditioned by a simple recessive factor. In the Clemson Nonshatter x Lincoln and Clemson Nonshatter x Ogden crosses, multiple secondary factors for resistance were also evident. Apparently, these secondary factors for resistance were contributed by Lincoln and Ogden and account for their being less susceptible than Boone.
9. There was no evidence of any linkage relations between the factors for resistance and maturity in the crosses Clemson Nonshatter x Lincoln and Clemson Nonshatter x Boone.

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